CAUSE OF LIME-INDUCED CHLOROSIS AND AVAILABILITY OF IRON IN THE SOIL

By P. L. GILE, *formerly Chemist*, and J. O. CARRERO, *Assistant Chemist, Porto Rico Agricultural Experiment Station*

CAUSE OF LIME-INDUCED CHLOROSIS

INTRODUCTION

Some years ago a study was made of a chlorosis of pineapples that occurred on certain soils in Porto Rico (*12*).[1] The particular type of chlorosis was confined to calcareous soils and seemed to be induced by a disturbance in the mineral nutrition of the plant. This disturbance appeared to consist in a lack of iron in the plant ash or in a diminished amount of iron combined with an increased amount of lime. Considerable work has since been carried on to determine more exactly the manner in which carbonate of lime in the soil induces chlorosis in the plant. The work comprises a number of direct experiments on the cause and cure of chlorosis as well as general studies in plant nutrition undertaken to gain information necessary for interpreting results obtained in the experiments on chlorosis. Since the more general work on plant nutrition has been published elsewhere, only the results will be referred to here.

In the following pages the more important facts already established concerning the cause of lime-induced chlorosis are given, together with a full report of certain experiments on this subject hitherto unpublished.

EVIDENCE THAT CARBONATE OF LIME MAY INDUCE CHLOROSIS

Evidence that carbonate of lime produces chlorosis in certain plants naturally falls into two classes, the results of soil surveys and the results of direct tests with natural or artificial calcareous soils. These two classes of evidence will be considered separately.

RESULTS OF SOIL SURVEYS

ECOLOGICAL STUDIES OF CALCIPHILOUS AND CALCIFUGOUS PLANTS.—Under the heading of soil surveys, reference should be made to the extensive literature on calciphilous and calcifugous plants. This literature, of which Roux (*39*) gives a complete bibliography up to 1900, consists chiefly of observations concerning the confinement of certain plants to calcareous or noncalcareous soils. While most of these

[1] Reference is made by number (italic) to "Literature cited," p. 59-61.

Journal of Agricultural Research, Washington, D. C.
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Vol. XX, No. 1
Oct. 1, 1920
Key No. B-16

observations do not deal directly with chlorosis, all are related to this subject, since calcifugous plants are often chlorotic on calcareous soils and since an exposition of the causes of chlorosis may afford an explanation of the calcifugous character of some plants.

There are a few plants which are very generally classed as calcifugous. Among these are the following: Maritime pine (*Pinus pinaster*) (*9*) chestnut (*Castanea vesca*), blueberry (Vaccinium), yellow and blue lupines (*Lupinus luteus* and *L. angustifolius*), certain species of sphagnum moss, etc. Cases have been recorded, however, where some plants generally considered calcifugous have been found growing on calcareous soils (*7*).

Probably the unsuitability of calcareous soils for certain plants is due not to carbonate of lime itself but to some soil characteristic usually associated with carbonate of lime. This being so, calcifugous plants might occur on certain calcareous soils provided some factor were operating to counteract the inhibiting characteristic usually associated with carbonate of lime.

STUDIES OF CHLOROTIC PLANTS.—Besides the soil surveys of calcifugous plants, there are several soil surveys which deal directly with the appearance of chlorosis in cultivated plants.

A case that has been the subject of much study is that of European grapes grafted on certain American stocks. When these were introduced on the calcareous soils of France and Germany they became chlorotic. Several soil surveys and many observations prove that the chlorosis is confined to calcareous soils and that there are varietal differences among grapes with respect to their resistance to lime (*22, 30, 33, 39 Viala and Ravaz, 45*). The accumulated data do not show, however, that all soils containing more than a certain percentage of carbonate of lime produce chlorosis in these varieties of grapes.

The chlorosis and failure of chestnut trees on most soils containing more than 3 per cent of carbonate of lime has been well established through soil surveys and through observations by Fliche and Grandeau (*10*), Piccioli (*36*), Vallot (*44*), and others. Vallot (*44, p. 202*) states that Dr. Bonnet reported that the chestnut failed to grow in a calcareous soil of Dijon, but when it was grafted on an oak it grew superbly.

That yellow and blue lupines and serradella become chlorotic when planted on calcareous soils is common knowledge in the calcareous districts of France and Germany, 2 per cent of carbonate of lime usually being sufficient to affect these plants.

A soil survey in Porto Rico showed that a chlorosis of pineapples was confined to the calcareous soils (*12, p. 8–18*). The only calcareous soils not producing chlorotic pineapples on which data could be obtained were some from the Florida Keys. These contained an exceptional amount of organic matter.

A chlorosis of sugar cane in Porto Rico was also found to be confined to calcareous soils, although very many calcareous soils did not induce chlorosis. Green cane was found growing on a soil containing 76.70 per cent calcium carbonate (*19*).

Pears have frequently been reported as showing chlorosis on calcareous soils (*4, 6, 29, 38*).

Instances have been noted where a great many other plants have become chlorotic on calcareous soils (*24*). Many of these cases are doubtless more or less exceptional, since some of the plants do not become chlorotic on most calcareous soils. Roux (*39*), without attempting a complete compilation, mentions some 50 genera and species of cultivated plants, ranging from mosses and orchids to maples and citrus trees, which have shown chlorosis when planted on soils containing calcium carbonate.

The results of the soil surveys and field observations seem to demonstrate conclusively that this type of chlorosis is confined under field conditions to calcareous soils. Probably no one species of plant, however, becomes chlorotic on all soils containing more than a certain percentage of calcium carbonate. Some plants are much more sensitive to carbonate of lime than others—that is, they become chlorotic on soils with lower lime contents and are less frequently found growing normally on limy soils.

The fact that plants very subject to chlorosis have been found in a few instances growing normally on markedly calcareous soils shows that the ability of calcareous soils to induce chlorosis does not depend entirely on the percentage of carbonate of lime in the soil. This fact also lends credence to the idea that it is not the carbonate of lime itself that induces chlorosis but some condition usually associated with the presence of carbonate of lime.

RESULTS OF VEGETATIVE EXPERIMENTS IN WHICH CHLOROSIS WAS PRODUCED BY NATURAL OR ARTIFICIAL CALCAREOUS SOILS

Compared with the mass of observations on the natural occurrence of chlorosis, there has been little reported in regard to inducing chlorosis by the use of calcium carbonate or in regard to direct tests of calcifugous plants in calcareous soils. There have been several vegetative experiments with yellow and blue lupines, however, where the addition of carbonate of lime to the soils caused a marked depression in growth and, in some cases at least, induced chlorosis. Concordant, positive results were secured by Heinrich (*23*), Meyer (*32*), Pfeiffer, and Blanck (*35*), the Agricultural Chemical Experiment Station at Breslau (*2*), Creydt (*5*), and Roux (*39, p. 147–185*).

Büsgen (*3*) grew the calcifugous broom (*Sarothamnus scoparius*), foxglove (*Digitalis purpurea*), and heather (*Calluna vulgaris*) in artificial calcareous and noncalcareous soils. The growth of all three plants was moderately to greatly depressed in the calcareous soil, although only broom was mentioned as showing chlorosis.

Roux (*39, p. 147*) grew some 20 species of calcifugous plants in calcareous soils. All species made diminished growth and became chlorotic in certain calcareous soils, while none showed chlorosis in the noncalcareous soil.

Piccioli (*36*) planted many varieties of chestnuts, together with Sarothamnus, Calluna, and Pteris, on soils with different additions of carbonate of lime. Most plants eventually died on the soil containing 12 per cent calcium carbonate.

Experiments at this Station showed that the mere addition of carbonate of lime to soils which normally produced green pineapples (*12, p. 20*) or rice plants (*13, p. 30*) caused the soils to produce chlorotic plants.

The preceding experiments seem to afford direct proof of the conclusions derived from field observations and from soil surveys that a chlorosis of some plants is caused by, or associated with, the presence of carbonate of lime in the soil.

MANNER IN WHICH CARBONATE OF LIME IN THE SOIL INDUCES CHLOROSIS IN THE PLANT

While it is quite generally conceded that carbonate of lime may induce a chlorosis in certain plants, there is a great diversity of ideas regarding the way the chlorosis is brought about. There are several classes of evidence or kinds of data on which conclusions concerning the nature of lime-induced chlorosis are based. These different kinds of evidence will be considered under the following heads: Evidence from analyses of plant ashes, effect of application of iron salts, effect of other lime compounds in inducing chlorosis, and effect of an alkaline reaction in inducing chlorosis.

RESULTS OF ASH ANALYSES OF PLANTS

In their work on the chlorosis of the chestnut and maritime pine Fliche and Grandeau (*9, 10*) analyzed leaves and branches of green and chlorotic trees. They concluded that the chlorosis and diminished growth of the trees on the calcareous soils were the result of an undue absorption of lime and a diminished absorption of other elements, notably potash and iron.

Schulze (*42*) analyzed the wood and leaves of green and chlorotic grapevines,[1] determining only lime, magnesia, potash, and soda. Compared with the green plants, the chlorotic ones had about one-half as much potash and soda and slightly more lime and magnesia in the ash.

Büsgen (*3*) analyzed the broom plants grown by him in calcareous and noncalcareous soils to determine lime and potash. The chlorotic and

[1] Analyses by Mach and Kurmann (*31*) are often quoted in this connection. The results probably have no bearing on this subject, however, as the chlorosis of their specimens seems to have been caused by too much moisture or poor drainage.

green plants from the two soils had almost equal percentages of lime and potash in the ash, the percentage of total ash in the dry substance being higher in the chlorotic plants.

Numerous ash analyses were made at this Station from chlorotic and green pineapple plants grown in soils with and without carbonate of lime (*12*). Compared with the green plants, the chlorotic ones in the calcareous soils contained more lime and less iron in the ash; differences in other ash constituents were slight or inconstant, potash as a rule being fully as high in the chlorotic plants as in the green ones.

Green and chlorotic rice plants were also analyzed at different ages for their mineral constituents (*13*). In the case of rice grown 25 days, the chlorotic plants from the calcareous soils contained much more lime, less iron, and equal or greater percentages of potash in the ash than the green plants from the soil containing no carbonate of lime; but in the case of green and chlorotic rice of 84, 102, and 129 days' growth, the only constant difference in the ash of the two kinds of plants was a greater percentage of lime in the chlorotic plants. These analyses and a special study showed that the percentage of iron in the ash of rice diminished very markedly as the plants became more mature (*15*). Since plants affected with chlorosis matured much more slowly than normal plants, probably the iron contents of the 84-, 102-, and 129-day samples were affected more by the different maturities of the plants than by the character of the soils.

Four pairs of samples of green and chlorotic sugar-cane leaves were analyzed for their ash constituents. The leaves were selected from canes which were of the same size and age and which were growing on the same calcareous soil. In each case the chlorotic leaves had a distinctly lower percentage of iron in the ash than the corresponding green leaves (*19*).[1]

A summary of the evidence from ash analyses in regard to the cause of lime-induced chlorosis is as follows: Lime was determined in all seven species of plants analyzed by the different investigators, and in five cases it appeared that an excessive absorption of this element might be a cause of chlorosis; in two cases it appeared that it was not. Potash was determined in six of the different plants, and in only three cases did it appear that a lack of potash might be a cause of chlorosis. Iron was determined in five of the plants, and in all five cases it appeared that the chlorosis might be due to a deficiency of this element.

The weight of the evidence from the ash analyses seems to be that a deficiency of iron in the ash is at least one cause of the chlorosis and that possibly an excess of lime is also a cause. Against this conclusion there is the opinion of many physiologists, as Euler (*8*), Jost (*28*), and Sorauer

[1] In a fifth comparison, leaves of green, slightly chlorotic, and chlorotic cane were analyzed, the canes being of equal age but of markedly different size when grown on calcareous and noncalcareous soils. The chlorotic leaves contained very slightly more iron than the green leaves. In this case, it is believed that the maturities of the plants and the different ages of the leaves were the chief factors influencing the iron content (*19, p. 15*).

(*43*), that lime-induced chlorosis is caused chiefly by a lack of potash in the plant ash. This opinion is evidently based only on the analyses of Fliche and Grandeau (*9, 10, 11*) and on those of Schulze (*42*). If a lack of potash in the ash were the cause of the chlorosis, plants grown in noncalcareous soils and in water cultures, under controlled conditions, with an insufficient supply of potash, should show this type of chlorosis. In such cases, however, the lack of potash is indicated by the appearance of brown spots on the leaves and not by a yellowing.[1] However, it has not been shown that a combined excess of lime and deficiency of potash would not produce chlorosis.

The reliability of ash analyses as the sole means of diagnosing the cause of chlorosis is questionable. At the most, the results of ash analyses should be taken as merely indicating the cause or as confirming other evidence. The ash compositions of normal plants show such wide variations and are affected by so many conditions that it is sometimes unsafe to assume that of two lots of plants those which have made the better growth have an ash composition more nearly normal.

Aside from difficulties in properly interpreting the results of ash analyses, it is sometimes doubtful whether the samples selected for analysis are truly comparable, even when whole plants are taken. This uncertainty was demonstrated in the analyses of rice, previously referred to. The practice of taking only a portion of a plant for analysis is also susceptible to error, especially where iron is to be determined. Since iron appears to be relatively immobile in the plant after it is once transported to the leaves, certain leaves of a plant might contain a sufficiency of iron while other leaves and the plant as a whole might lack iron (*16*).

EFFECT OF APPLICATION OF IRON SALTS TO CHLOROTIC PLANTS

Eusebe Gris, in 1845 (*20*), and later Sachs (*41*) and other investigators (*12, 21, 25, 26, 27*) showed that various plants which became chlorotic on calcareous soils could be cured by applying ferrous sulphate to the leaves. This treatment and the improved one of Rassiguier (*37*), that of brushing cut surfaces of pruned vines with a concentrated solution of ferrous sulphate, have been rather generally used on grapevines which became chlorotic on the calcareous soils of France and Germany.

Various investigators have found that while iron salts were effective in overcoming chlorosis when applied to the stems and leaves of plants, they were ineffective when applied to the soil, even if used in considerable quantity. Sachs (*41*), however, observed that where the roots of plants were not completely surrounded by earth, as in the case of pot-bound plants, applications of ferrous sulphate to the soil did cure the chlorosis.

[1] If potash is concerned in the formation of starch from sugars, a low percentage of potash in chlorotic plants might be a secondary result of the chlorosis. With insufficient iron, chlorophyll formation is depressed, less sugar can be synthesized, and little potash would be needed.

Since ferrous sulphate is, of course, immediately transformed into ferric carbonate in a calcareous soil, it seems evident that calcium carbonate renders ferric carbonate unavailable, or less available, to certain plants.

It has been repeatedly demonstrated that the effectiveness of spraying with ferrous sulphate is due only to the iron and that only soluble iron salts are effective (*12, 21, 25, 26, 27*).

EXPERIMENT I.—The results in Table I show the effect of an iron spray upon chlorotic rice growing in a calcareous soil. The plants were grown in the open from February 29 to July 13, 1912, in small brick compartments with 36 plants to each compartment. Each compartment held about 200 pounds of heavy loam soil and received 5 gm. nitrogen, 3.4 gm. phosphoric acid, and 5 gm. potash, derived from various commercial fertilizers. The plants sprayed with ferrous sulphate were given 4 applications of a 0.5 per cent solution and 12 applications of a 1 per cent solution.

TABLE I.—*Effect of an iron spray upon chlorotic rice plants grown on calcareous soils*

Test No.	Calcium carbonate content of soil.	Treatment of plants.	Green weight of plants per compartment.		
			Series A.	Series B.	Average.
	Per cent.		*Gm.*	*Gm.*	*Gm.*
1	0	Unsprayed	1,022	1,071	1,047
2	0	Sprayed with ferrous sulphate	1,088	1,040	1,064
3	30	Unsprayed	4	([a])	....
4	30	Sprayed with ferrous sulphate	946	894	920
5	50	Unsprayed	242	702	472
6	50	Sprayed with ferrous sulphate	874	893	884

[a] Some plants were eaten by mole cricket, but according to comparative growths of plants before any were eaten, the weight would have been about 250 gm.

Twenty-one days after planting, the plants in the calcareous soils were markedly chlorotic, and spraying was begun at that time. Seven days later, after nine sprayings, the sprayed plants in the calcareous soils were much superior to the unsprayed in color and growth. All plants in the noncalcareous soil had a good color at all times. (Pl. 5, A.)

The results obtained by treating the leaves and stems of chlorotic plants with iron salts show clearly that a lack of iron in the plant is at least one of the causes of lime-induced chlorosis. This conclusion is substantiated by the results of ash analyses of the plants. But this work does not show: (1) whether the lack of iron in the plant is due to a low availability of iron in the soil or to reactions in the plant rendering ineffective the iron absorbed; (2) whether an increased absorption of lime is a contributory cause of chlorosis; or (3) whether the reaction of the soil has any effect on the appearance of chlorosis, aside from affecting the iron supply.

EFFECT OF COMPOUNDS OF LIME IN INDUCING CHLOROSIS

To see whether lime salts in general induce chlorosis in certain plants, experiments have been conducted with calcium carbonate, sulphate, phosphate, and silicate. The effects of these compounds on the growth of lupines have been determined by Heinrich (*23*), Pfeiffer and Blanck (*35*), and Creydt (*5*). The calcium sulphate did not induce chlorosis but depressed growth considerably, although much less than the calcium carbonate, while calcium phosphate and silicate were markedly toxic. The toxicities of the latter two substances were attributed to their acid and alkaline reactions, respectively.

Large quantities of gypsum depressed the growth of pineapples about 20 per cent but did not cause chlorosis (*12*). Various experiments were conducted to determine the effect on rice of large amounts of assimilable lime in the form of gypsum.

EXPERIMENT II.—In this experiment, rice plants were grown from December 17, 1912, to May 20, 1913, in small brick compartments, with 24 plants to each compartment. Each compartment held about 200 pounds of soil fertilized with 30 gm. sulphate of ammonia, 20 gm. nitrate of soda, 30 gm. acid phosphate, and 18 gm. muriate of potash added in two applications. The results are shown in Table II.

TABLE II.—*Effect on the growth of rice of adding gypsum to the soil*

Test No.	Kind of soil.	Gypsum ($CaSO_4.2H_2O$) added.	Green weight of plants per compartment.			
			Series A.	Series B.	Series C.	Average.
		Per cent.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	Loam	0	1,218	1,229	1,452	1,300
2	.....do	15	312	446	382	380
3	Clay	0	....	808	840	824
4	.....do	15	936	958	842	912

During the first four weeks the plants were all of good color, but later the plants in the loam soil containing gypsum became yellow, though not typically chlorotic.

EXPERIMENT III.—A second experiment was conducted to see whether large amounts of gypsum would depress the growth of rice if the plants were sprayed with ferrous sulphate. The compartments contained about 200 pounds of a sandy soil and received 45 gm. sulphate of ammonia, 30 gm. acid phosphate, and 18 gm. muriate of potash. In each compartment 22 plants were grown. The plants treated with ferrous sulphate were sprayed twice with a 0.1 per cent solution, five times with a 0.15 per cent solution, once with a 0.2 per cent solution, three times with a 0.75 per cent solution, and seven times with a 1 per cent solution. The results are given in Table III.

TABLE III.—*Influence of spraying with ferrous sulphate on the depressing effect of gypsum*

Test No.	Gypsum ($CaSO_4 . 2H_2O$) added.	Treatment of plants.	Air-dried weight of plants per compartment.			
			Series A.	Series B.	Series C.	Average.
	Per cent.		*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	0	Unsprayed	521	503	475	500
2	0	Sprayed with ferrous sulphate.	478	525	525	509
3	15	Unsprayed	324	300	366	330
4	15	Sprayed with ferrous sulphate.	395	364	372	377

The plants in the soil to which gypsum had been added were markedly behind the others in growth from the start, and they were at times of poorer color, though they were never typically chlorotic. Plants in soil without gypsum were of good color at all times. No effect from spraying with ferrous sulphate was observable.

EXPERIMENT IV.—A further experiment with gypsum and ferrous sulphate was conducted in pots in a glass house. Six rice plants per pot were grown from July 7 to October 25, 1913. Each pot contained 37 pounds of sandy soil, to which 13 gm. ammonium sulphate, 11 gm. acid potassium phosphate, and 3.6 gm. sulphate of potash were applied. The moisture content of the soil was maintained at the optimum. The results appear in Table IV.

TABLE IV.—*Influence of different treatments with ferrous sulphate on the depressing effect of gypsum*

Test No.	Gypsum ($CaSO_4 . 2H_2O$) added.	Treatment of plants.	Air-dried weight of plants per pot.				
			Series A.	Series B.	Series C.	Series D.	Average.
	Per cent.		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	0		114	113	113	118	115
2	15		85	92	68	67	78
3	15	Ferrous sulphate, 2.2 gm., added to soil.		50	86	85	74
4	15	Plants sprayed eight times with 1 per cent solution of ferrous sulphate.	67	60	60	78	66

The color of the plants grown in the soil to which gypsum was added was as good as that of the controls up to the eighty-fifth day, but from the eighty-fifth to the one hundred and tenth day the former were yellow. The controls were always of a good green color. No effect from either of the treatments with ferrous sulphate was observable.

SUMMARY.—In all the tests, except that with the clay soil, calcium sulphate depressed the growth of rice and induced a certain amount of yellowing. The yellowing, however, was not that typical of lime-induced chlorosis. Spraying with ferrous sulphate and adding ferrous sulphate to the soil failed to increase the growth or improve the color of plants growing in the soil containing calcium sulphate. That calcium sulphate increased the amount of lime in the plants may be seen by the analyses in Table V of plants 65 days old from experiment II.

TABLE V.—*Ash analyses of plants from experiment II*

Test No.	Kind of soil.	Gypsum ($CaSO_4.2H_2O$) added.	Carbon-free ash in dry substance of plants.	Analyses of carbon-free ash.							
				Silica (SiO_2).	Lime (CaO).	Magnesia (MgO).	Potash (K_2O).	Soda (Na_2O).	Iron (Fe_2O_3).	Phosphoric acid (P_2O_5).	Sulphur (SO_3).
		P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
1	Loam..	0	16.75	54.70	3.63	4.20	24.77	5.48	0.55	2.58	2.19
2	...do...	15	13.92	47.24	6.45	5.22	26.81	4.54	.53	4.01	6.71
3	Clay...	0	14.14	50.77	3.87	4.80	25.11	4.65	.62	2.31	2.74
4	...do...	15	13.08	45.04	6.40	4.77	28.34	7.00	.38	3.13	4.76

It will be noted that the calcium sulphate increased the percentages of lime and sulphur in the plant ash and diminished the percentage of silicia but had little effect on the other constituents.

The injurious effect of calcium sulphate on rice might have been due to several causes. A large amount of gypsum evidently maintains a solution more concentrated than that existing in any except alkali soils. There is also the possibility of hydrogen sulphid being formed by bacteria reducing sulphates. This occurred with soil preserved in a sample jar, although such a result was not to be expected in what appeared to be a normally aerated soil. The fact that calcium sulphate did not depress growth in the clay soil lends credence to the view that the injurious effect might have been that of a too concentrated soil solution.

In order to make sure that an increased assimilation of lime is not a cause of chlorosis, a test was conducted with lime salts applied to the leaves. The results, given in experiments V and VI, to be described further on, seemed to show definitely that an increased assimilation of lime does not induce chlorosis.

Although excessive quantities of various lime compounds seem to be more or less injurious, each one appears to act differently; there is no evidence of a general "lime effect" in inducing chlorosis.

EFFECT OF AN ALKALINE REACTION IN INDUCING CHLOROSIS

Pfeiffer and Blanck (*35*) in their first work on the intolerance of lupines for calcareous soils concluded that lupines are especially sensitive to an alkaline reaction and that the carbonate of lime not only depresses the absorption of nutrients but is directly injurious to the roots of the plants. While the alkaline reaction of carbonate of lime is evidently a factor in the chlorosis, it is very evidently not directly injurious to roots of even calcifugous plants. It was found in experiments with pineapple and rice at this Station that the ratio of root to top growth was much increased in calcareous soils and solutions (*12, 17*). The stimulating effect of carbonate of lime on the root growth of plants which are not calcifugous has been frequently noted.

In work with pineapples it was shown that the alkalinity induced by increasing amounts of carbonate of soda greatly depressed growth without affecting the color of the plants (*12, p. 31*).

Work with rice in water cultures seemed to show definitely that the alkalinity of carbonate of lime is not directly injurious to this calcifugous plant, nor is the alkalinity in itself the cause of chlorosis (*17*). Rice was grown with different quantities of iron from different sources in nutrient solutions which were acid, neutral, and alkaline from carbonate of lime. A summary of the relative growths made under the different conditions is given in Table VI.

TABLE VI.—*Relative growths of rice plants with different amounts of iron from various sources in acid, neutral, and alkaline solutions*

Source of iron in nutrient solutions.	Iron per liter added to nutrient solutions.	Relative growths in—		
		Acid solution.	Neutral solution.	Alkaline solution.
	Gm.			
Ferrous sulphate	0.002	100	88	....
Do	....	100	74	51
Do	....	100	95	95
Do. Do.	.008 .004	100	105	....
Do	.002	100	107	....
Do	.008	100	132	....
Do	....	100	111	2
Ferric chlorid	.002	100	99	26
Do	.008	100	107	26
Ferric citrate	.002	100	85	86
Do	....	100	94	97
Do	....	100	101	104
Do	.008	100	85	58
Ferric tartrate	.002	100	80	76
Do	.008	100	96	100
Dialyzed iron	.008	100	27	....

Where growth was depressed to any extent the plants were more or less chlorotic, and that this chlorosis was evidently due to lack of iron was shown by analyses of the plants and by treatment of the leaves with ferrous sulphate. The work showed quite definitely that rice is not particularly sensitive to the reaction of carbonate of lime, except as the reaction influences the availability of the iron. When the proper form of iron was used in the proper quantity, the growth and appearance of the plants were as good in the solutions containing carbonate of lime as in the acid or neutral solutions.

The preceding results seem to show that neither increased assimilation of lime nor mere alkalinity causes chlorosis. It remained to be seen

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whether the combination of the two conditions would induce a typical chlorosis. It was thought that this might be determined experimentally by growing rice on soil to which sodium bicarbonate had been added to render it alkaline and then inducing an increased assimilation of lime by spraying the plants with calcium chlorid and gypsum. In case these treatments should induce a chlorosis identical with that produced by carbonate of lime, spraying with ferrous sulphate should cure it. Accordingly, some plants grown in the soil with sodium bicarbonate were sprayed with lime salts alone, with ferrous sulphate alone, and with both lime and iron salts. Plants grown in a soil without sodium bicarbonate were also sprayed as described above in order to check the results.

The experiment was carried out twice, once in the open, using small brick compartments, and once in the glass house, using pots. Results are given under the heads of experiments V and VI. The sodium bicarbonate was added in several doses until it became evident that sufficient had been applied to affect growth. More was required for the soil in the open than for that in the glass house, since the former was exposed to leaching.

EXPERIMENT V.—This test was run from November 8, 1913, to January 20, 1914. Each plot containing 150 pounds of sandy soil received 45 gm. sulphate of ammonia, 30 gm. acid phosphate, and 18 gm. muriate of potash. Thirty rice plants were grown on each plot. The results are given in Table VII.

TABLE VII.—*Effect of sodium bicarbonate, lime, and iron on the growth of rice: Experiment V*

Test No.	Approximate percentage of sodium bicarbonate in soil.	Treatment.	Air-dried weight of plants per plot.		
			Series A.	Series B.	Average.
			Gm.	*Gm.*	*Gm.*
1	0	None	144	156	150
2	0	Sprayed 31 times with 0.5 to 2 per cent solutions of calcium chlorid and gypsum	142	152	147
3	0	Sprayed 31 times with 0.5 to 2 per cent solutions of calcium chlorid and gypsum and 8 times with 0.5 to 1 per cent solutions of ferrous sulphate	163	166	165
4	0.2	None	100	104	102
5	.2	Sprayed 31 times with 0.5 to 2 per cent solutions of calcium chlorid and gypsum	101	82	92
6	.2	Sprayed 31 times with 0.5 to 2 per cent solutions of calcium chlorid and gypsum and 8 times with 0.5 to 1 per cent solutions of ferrous sulphate	110	100	105
7	.2	Sprayed 8 times with 0.5 to 1 per cent solutions of ferrous sulphate	......	113	113

The plants in the soils containing sodium bicarbonate became somewhat yellow, though the yellowing was not that of typical lime-induced chlorosis. The yellowing, however, was not increased by the lime spray, nor was it overcome by the iron spray. The lime and iron sprays also had no effect on the appearance of the plants growing in the soil containing no sodium bicarbonate.

EXPERIMENT VI.—In this test, conducted from November 4, 1913, to March 12, 1914, 7 rice plants were grown per pot. Each pot contained 35 pounds of sandy soil and received 6 gm. ammonium nitrate, 1.3 gm. potassium acid phosphate, and 2.5 gm. potassium sulphate. The moisture content was maintained at 25 per cent of the dry weight of the soil. The results are shown in Table VIII.

TABLE VIII.—*Effect of sodium bicarbonate, lime, and iron on the growth of rice: Experiment VI*

Test No.	Approximate percentage of sodium bicarbonate in soil.	Treatment.	Air-dried weight of plants per pot.		
			Series A.	Series B.	Average.
			Gm.	*Gm.*	*Gm.*
1	0	None	83	71	77
2	0	Plants sprayed 23 times with 0.5 to 2 per cent solutions of calcium chlorid and sulphate	68	72	70
3	0	Plants sprayed 23 times with 0.5 to 2 per cent solutions of calcium chlorid and sulphate and 7 times with 0.5 to 1 per cent solutions of ferrous sulphate	65	70	68
4	0.08	None	44	56	50
5	.08	Plants sprayed 23 times with 0.5 to 2 per cent solutions of calcium chlorid and sulphate	52	51	50
6	.08	Plants sprayed 23 times with 0.5 to 2 per cent solutions of calcium chlorid and sulphate and 7 times with 0.5 to 1 per cent solutions of ferrous sulphate	42	39	40
7	.08	Plants sprayed 7 times with 0.5 to 1 per cent solutions of ferrous sulphate	49	60	55

The appearance of the plants in this test was the same as in experiment V.

The plants from experiment V were analyzed for their ash constituents, and the results appear in Table IX. The plants were washed immediately after cutting, so no lime salts remained on the leaves. While it is believed that all iron applied as a spray was also removed by washing, it is possible that some iron in the form of ferric oxid might have remained adhering to the leaves; hence, in the case of the plants sprayed with ferrous sulphate, it is possible that the analytical results may show more iron than was actually present in the plants.

TABLE IX.—*Analyses of rice plants growing in a soil with sodium bicarbonate and sprayed with lime and iron salts*

CONSTITUENTS OF ASH IN TOTAL DRY MATTER

Test No.	Approximate percentage of sodium bicarbonate in the soil.	Treatment.	Carbon-free ash.	Silica (SiO_2).	Lime (CaO).	Magnesia (MgO).	Potash (K_2O).	Soda (Na_2O).	Iron (Fe_2O_3).	Phosphoric acid (P_2O_5).	Nitrogen (N).
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	0	None	11.17	4.51	0.48	0.59	3.75	0.30	0.015	0.72	2.57
2	0	Plants sprayed with 0.5 to 2 per cent solutions of calcium chlorid and sulphate	14.12	6.83	.83	.64	4.31	.35	.021	.62	2.57
3	0	Plants sprayed with 0.5 to 2 per cent solutions of calcium chlorid and sulphate and 0.5 to 1 per cent solutions of ferrous sulphate	12.80	5.88	.71	.39	3.30	.47	.065	.69	2.74
4	0.2	None	14.98	8.06	.54	.82	3.44	1.02	.018	.69	2.80
5	.2	Plants sprayed with 0.5 to 2 per cent solutions of calcium chlorid and sulphate	15.56	7.84	1.10	.70	3.44	.88	.019	.66	2.91
6	.2	Plants sprayed with 0.5 to 2 per cent solutions of calcium chlorid and sulphate and 0.5 to 1 per cent solutions of ferrous sulphate	14.96	7.78	.81	.66	3.43	.73	.084	.68	2.73
7	.2	Plants sprayed with 0.5 to 1 per cent solutions of ferrous sulphate	12.41	5.98	.51	.68	3.20	.91	.063	.64	....

CONSTITUENTS OF CARBON-FREE ASH

Test No.	Approximate percentage of sodium bicarbonate in the soil.	Treatment.	Carbon-free ash.	Silica (SiO_2).	Lime (CaO).	Magnesia (MgO).	Potash (K_2O).	Soda (Na_2O).	Iron (Fe_2O_3).	Phosphoric acid (P_2O_5).	Nitrogen (N).
1	0	None	....	40.42	4.29	5.27	33.61	2.72	0.14	6.44	....
2	0	Plants sprayed with 0.5 to 2 per cent solutions of calcium chlorid and sulphate	....	48.46	5.87	4.50	30.48	2.45	.15	4.39	....
3	0	Plants sprayed with 0.5 to 2 per cent solutions of calcium chlorid and sulphate and 0.5 to 1 per cent solutions of ferrous sulphate	....	46.01	5.53	3.04	29.72	3.70	.51	5.41	....
4	0.2	None	....	53.90	3.60	5.46	22.95	6.80	.12	4.59	....
5	.2	Plants sprayed with 0.5 to 2 per cent solutions of calcium chlorid and sulphate	....	50.34	7.07	4.52	22.18	5.65	.12	4.26	....
6	.2	Plants sprayed with 0.5 to 2 per cent solutions of calcium chlorid and sulphate and 0.5 to 1 per cent solutions of ferrous sulphate	....	52.01	5.74	4.44	22.96	4.91	.56	4.54	....
7	.2	Plants sprayed with 0.5 to 1 per cent solutions of ferrous sulphate	....	48.13	4.10	5.46	25.77	7.30	.51	5.18	....

SUMMARY.—None of the sprays affected the growth or color of the plants, either in the normal soil or in the soil containing sodium bicarbonate. The amount of sodium bicarbonate required to depress growth was rather surprising, and from this fact it was suspected that the availability of iron was not noticeably depressed by sodium bicarbonate, at least not below the critical point. This was confirmed by the analyses of the plants and by the fact that spraying with ferrous sulphate effected no improvement in either the growth or color of the plants planted in the soil containing sodium bicarbonate.

The spraying with lime salts, however, notably increased the amount of lime in the plants without affecting the quantity of iron, and spraying with both lime and iron solutions increased the quantities of both elements in the plant. The yellowing and depression in growth produced by the sodium bicarbonate were probably due to an injurious degree of alkalinity, which must have been far greater than that which is produced by carbonate of lime.

The results of these experiments, where a large amount of sodium bicarbonate was required to depress growth, seem to show that the slight alkalinity of carbonate of lime could not be directly injurious to rice, nor could alkalinity in itself be the cause of chlorosis. While this experiment failed to yield the decisive answer expected, it is felt that the results point strongly to the conclusion that an increased assimilation of lime is not the cause of chlorosis.

CHLOROSIS DUE SIMPLY TO A DEPRESSION IN AVAILABILITY OF IRON IN THE SOIL

An attempt was made to demonstrate directly that the only action of carbonate of lime in inducing chlorosis lies in depressing the availability of the iron. It was thought that this demonstration could be accomplished by growing rice plants with their roots divided between two kinds of soil, one to contain carbonate of lime and all the mineral nutrients except iron, and the other to contain only iron. The attempt was not completely successful, due partly to a principle discovered later and partly to difficulties in execution. The principle which tended to make the results less striking than had been anticipated is the following: Plants apparently are unable to attain a maximum absorption of any one element with only a part of their roots (*18*).

Wire sieves were made which fitted into the tops of buckets. The buckets were filled with soil to within 1 inch of the bottom of the sieves, and the sieves were filled with about 2 inches of soil (Pl. 5, B). In this way an air space was left between the soil in the sieve and that in the bucket; this prevented any soil solution passing by capillary attraction from the soil below to that above. It was the intention at first to fill all except the control buckets with a calcareous soil containing all the nitrogen, phosphoric acid, and potash, and to fill most sieves with pure silica sand containing only iron. In conducting the experiment, however, it was found necessary to apply a small amount of nitrogen, phosphoric acid, and potash to the sand in the sieve in order that the plants might develop sufficiently for their roots to penetrate the soil below.

The intention was that the plants should absorb practically all their nutrients from the soil in the buckets (a calcareous soil except in control buckets 1 to 4), but that in some cases the plants should be able to absorb iron from a lime-free medium in the sieve. If carbonate of lime affected the plants in any way except through depressing the absorption of iron, all plants should make equally poor growth; but if, on the other hand, the only action of the carbonate of lime lay in decreasing the availability of the iron, those plants that could draw iron from a medium containing no carbonate of lime should do much better than the others.

A preliminary test was run with two pots, No. 1 containing silica sand in the sieve and a calcareous soil in the bucket, and No. 2 containing silica sand plus carbonate of lime in the sieve and the same calcareous soil in the bucket as No. 1. Four gm. of ferrous sulphate were applied to both sieves (Pl. 6, A). The yields from pots No. 1 and 2 were respectively 169 gm. and 97 gm. of air-dried plants, the plants in No. 1 being green in color and those in No. 2 chlorotic.

EXPERIMENT VII.—The results of a more extended test are given in Table X. The plants were grown from October 22, 1912, to March 3, 1913. A large number of seeds were planted, but the plants in each pot were thinned to eight. The sieve of each pot contained 10 pounds of silica sand to which were added 0.45 gm. ammonium nitrate, 0.1 gm. acid potassium phosphate, and 0.2 gm. potassium sulphate. The bucket of each pot contained 23 pounds of soil and received 12 gm. ammonium nitrate, 3 gm. acid potassium phosphate, and 5.5 gm. potassium sulphate, in two applications. The moisture content of the soil was maintained at 31 per cent of the dry weight.

TABLE X.—*Effect of carbonate of lime in the soil on the availability of iron*

Pot No.	Treatment of soil in bucket.	Treatment of sand in sieve.	Green weight of plants per pot.				
			Series A.	Series B.	Series C.	Series D.	Average.
			Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1 to 4	None	None	206	171	204	.....	194
5 to 8	Calcium carbonate, 15 per cent.	None	137	224	156	161	170
9 to 12	.....do	Eight gm. ferrous sulphate in four applications.	204	174	236	187	200
13 to 16	.....do	Eight gm. ferrous sulphate in four applications; 15 per cent calcium carbonate.	112	97	115	84	102

At 15 days after sowing the seed all plants were chlorotic except those in pots 13 to 16, and many died because of their inability to establish roots in the soil in the bucket. At 121 days the plants of pots 1 to 4 and 9 to 12 were green, while those of No. 5 to 8 and 13 to 16 were strongly chlorotic.

The plants encountered some difficulty in establishing their roots in the soil in the buckets; the roots after passing through the sieve often grew for a time on the surface of the soil. This retarded growth considerably, but when the roots once penetrated the soil, growth became normal. At the end of the experiment the greater part of the roots were in the soil in the bucket, where practically all the fertilizer was located.

The final yields of the plants and the chlorotic appearance of certain plants during the latter stages of growth confirm the idea that the only effect of carbonate of lime in inducing chlorosis lies in depressing the availability of iron. The plants in pots No. 9 to 12 and those in No. 13 to 16 were exposed to the same conditions except that the plants in No. 9 to 12 were able to draw part of their iron from a medium containing no carbonate of lime; this difference was sufficient to double the growth of plants. The plants of No. 9 to 12 had to assimilate practically all their mineral nutrients, except iron, from the same calcareous soil as the plants of No. 13 to 16; hence, if the carbonate of lime induced chlorosis by depressing the availability of any nutrients other than iron, or if an increased assimilation of lime were a contributory cause of chlorosis, the yield from pots No. 9 to 12 should have been practically the same as from No. 13 to 16.

The only apparent contradiction in this demonstration of the cause of lime-induced chlorosis lies in the fact pots No. 5 to 8 yielded more than No. 13 to 16. Plants in pots No. 5 to 8 evidently secured less iron than those in No. 9 to 12, for they made less growth; but if the sand in the sieve had been really iron-free they should have made no more growth than plants No. 13 to 16. Later work showed that, although no iron was added to the sieves of No. 5 to 8, doubtless the silica sand contained enough iron to cause the unanticipated growth. In work with nutrient solutions it was found that rice practically satisfied its iron requirements in a solution containing no more than 1 part of truly soluble iron in 10,000,000 parts of solution (*17, p. 5*).

On repeating this experiment the same difficulties were encountered, but the relative growths made by the differently treated plants were similar to those in the preceding test.

AVAILABILITY OF IRON IN THE SOIL

INTRODUCTION

Since the preceding summary of facts and experiments seems to indicate that lime-induced chlorosis is simply the result of insufficient available iron in the soil, evidently a knowledge of conditions affecting the availability of iron in the soil is essential to a complete understanding of this chlorosis. If all the conditions affecting the amount of available iron in the soil were known, it would doubtless be possible to explain why some calcareous soils induce chlorosis when others do not; why in a sandy soil a smaller percentage of carbonate of lime is required to induce chlorosis than in a clay soil; why a calcareous soil that produces chlorotic plants at one time may not at another; and many other perplexing facts.

Since a method for determining the amount of available potash or phosphoric acid in the soil is still unknown, in spite of years of work, the prospect is not bright for even roughly determining the available iron by direct means; and to determine directly significant differences in amounts of available iron seems hopeless when plants obtain their iron from such exceedingly dilute solutions.

Soils which yield sufficient iron for the growth of plants may not show a detectable amount of iron in the water extract. In some cases the water extract of soils may show considerable iron, but the iron may be in a colloidal state and not in true solution. Colloidal iron was found unavailable for rice in water culture (*14*).

While there are great difficulties in the way of determining the small, significant quantities of soluble or available iron in the soil, it seems from the work of Morse and Curry (*34*), Ruprecht (*40*), and Abbott (*1*) that acid soils may contain much more soluble iron and aluminum than neutral or calcareous soils and may even contain an injurious amount of these compounds.

The following work on the availability of iron compounds is based on the assumption that the chlorosis and the poor growth of rice in the calcareous soils were caused by a lack of available iron. This assumption seems justified by the results presented in the first part of this report.

AVAILABILITY OF ORGANIC IRON COMPOUNDS

In work with pineapples it developed that in the presence of a great amount of organic matter a large amount of carbonate of lime was required to induce chlorosis (*12*). This suggested that in calcareous soils organic iron compounds might be more available than the inorganic, just as iron in solution as a complex ion is less completely precipitated by the usual reagents. The idea seemed substantiated by tests with rice in nutrient solutions containing carbonate of lime, where ferric tartrate furnished much more available iron than equivalent quantities of ferrous sulphate or ferric chlorid.

EXPERIMENT VIII.—Tests were accordingly conducted to determine the effect of various iron compounds and organic materials on the growth of rice in both calcareous and noncalcareous soils. In this experiment the effects of certain pure organic compounds of iron were compared with those of ferric chlorid and ferrous sulphate. A substance which may be called "ferric molasses" was also used. This was prepared by boiling together 2 parts of ferrous sulphate and 10 parts of a final molasses. It doubtless contained some ferric acetate, glucolate, laevulate, possibly other organic iron compounds, and considerable inorganic iron. As a control on the action of the "ferric molasses," the same quantity of molasses which had been similarly boiled without addition of iron was applied to two other lots of pots. To one of these lots ferrous sulphate was applied after the boiled molasses had been mixed with the soil in the pots designated as "molasses and ferrous sulphate" in Table XI.

Five rice plants were grown in each pot from September 28 to December 28, 1914. In the noncalcareous series each pot contained 14 pounds of loamy soil with the moisture content maintained at 23 per cent of the dry weight; and in the calcareous series each pot contained 14 pounds of loamy soil with the moisture content maintained at 27 per cent of the dry weight. The calcareous soil contained 17.8 per cent of carbonate of lime. A fertilizer consisting of 1.8 gm. ammonium nitrate, 4.2 gm. sodium nitrate, 3 gm. ammonium sulphate, 0.4 gm. acid potassium phosphate, 3.9 gm. acid phosphate, and 3.8 gm. potassium sulphate was added to each pot in four applications. The molasses and all the iron compounds were mixed with the soil before the rice was planted. The iron was applied at the rate of 0.25 gm. and the molasses at the rate of 6.25 gm. per pot. The results of the experiment are summarized in Table XI.

TABLE XI.—*Comparative availability to rice plants of organic and inorganic compounds of iron in a calcareous and noncalcareous soil: Experiment VIII*

Special additions to the soil.	Oven-dried yield of plants per pot.											
	Calcareous soil.						Noncalcareous soil.					
	Series A.	Series B.	Series C.	Series D.	Series E.	Average.	Series A.	Series B.	Series C.	Series D.	Series E.	Average.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm*
....	20	16	9	12	13	14	39	41	38	41	59	44
Ferric chlorid	16	18	7	8	15	13	......	......	......	......	......	......
Ferric tartrate	12	21	26	12	26	19	49	55	57	43	50	51
Ferric citrate	18	12	13	23	7	15	51	42	43	42	46	45
Ferric valerianate	11	18	8	7	10	11	49	58	51	44	48	50
Ferric benzoate	9	20	34	18	18	20	49	58	46	52	59	53
Molasses	7	5	2	2	3	4	52	54	41	50	54	50
"Ferric molasses"	18	7	22	15	14	15	42	44	55	50	60	50
Molasses and ferrous sulphate	6	9	4	5	4	6	40	49	54	49	56	50
Ferrous sulphate	16	13	21	15	7	14	......	......	......	......	......	......

Three weeks after planting, all plants in the noncalcareous soil were green, while many plants in the calcareous soil were slightly chlorotic. Those plants in the calcareous soil which received molasses alone or molasses with ferrous sulphate were markedly chlorotic (Pl. 6, B). During later growth the plants in noncalcareous soil remained green and those in calcareous soil became more chlorotic, some plants eventually dying from the top down.

In the noncalcareous soil none of the special compounds affected growth significantly, and in the calcareous soil none of the iron compounds proved efficient sources of iron, although possibly the ferric tartrate and benzoate increased growth slightly.

Molasses alone and molasses followed by ferrous sulphate depressed growth markedly and intensified the chlorosis of plants in the calcareous soil, but the "ferric molasses" had no effect. Probably the molasses that had not been treated with iron still further depressed the availability of iron in the calcareous soils by promoting the formation of insoluble organic iron compounds.

EXPERIMENT IX.—Later a second test was conducted with pure organic iron compounds and organic materials containing iron in calcareous and noncalcareous soils. The pure iron compounds were applied so as to furnish 0.75 gm. or 1.50 gm. of iron per pot, the smaller application being at approximately the same rate as in the preceding experiment, if the sizes of the pots and quantities of soil used in the two experiments are considered. In the tests with ferric citrate and ferric tartrate, a comparison was made between the results obtained by mixing all the material with the soil before planting and those obtained by applying the material in small doses in solution during the growth of the plants. This was done to see if the materials might not be available for a short time in the soil although rendered unavailable in the course of time by bacterial or other action.

The "ferric humate," which, it was thought, might contain some iron compounds similar to those existing in a natural soil, was prepared by extracting leaf mold with 4 per cent ammonia, acidifying with hydrochloric acid, washing the precipitate free from chlorids, and evaporating the precipitate to dryness with sufficient ferric chlorid solution to furnish 25 per cent as much iron as dry matter. The "mixture" used per pot was composed of 4 gm. dried blood, 40 gm. Stizolobium vines, 40 gm. tobacco stems, and 0.90 gm. iron from equal parts of ferric citrate, tartrate, "humate," tannate, oxalate, and benzoate. Velvet beans (Stizolobium) were tested because they are extensively grown as a green manure crop. Both Stizolobium vines and tobacco stems were cut up before mixing with the soil. Citric and tartaric acids were tried to see whether an organic radical alone would have any effect in maintaining available iron in the soil. The test was conducted from December 8, 1916, to February 19, 1917, with eight rice plants in each pot. The pots contained 42 pounds of sandy loam soil, or 47 pounds of sandy soil containing 10 per cent carbonate of lime. The moisture contents of both soils were maintained at 18 per cent of the dry weight. The fertilizer for each pot was given in two applications and consisted of 15 gm. ammonium sulphate, 19.5 gm. acid phosphate, and 6 gm. potassium sulphate. The special additions were mixed with the top 4 inches of soil before the rice was planted, except the solutions of ferric citrate and ferric tartrate which were applied to the soil every other day. Results of the test are given in Table XII.

TABLE XII.—*Comparative availability to rice plants of organic and inorganic iron compounds in calcareous and noncalcareous soils: Experiment I*

Special additions to the soil.	Amount added.	Oven-dried yield of plants per pot.											
		Calcareous soil.						Noncalcareous soil.					
		Series A.	Series B.	Series C.	Series D.	Series E.	Average.	Series A.	Series B.	Series C.	Series D.	Series E.	Average.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
None	....	28	22	23	26	26	......	60	67	68	65	75	......
None	....	30	24	17	24	28	25	69	72	70	69	76	69
Ferric oxalate	2.43	18	21	20	24	24	21	78	70	66	54	75	69
Do	4.86	19	20	25	19	24	21	62	66	69	68	65	66
Ferric tannate	8.18	19	22	22	29	25	23	72	68	68	73	69	70
Do	16.36	23	28	23	22	28	25	69	68	77	68	75	71
"Ferric humate"	3.75	18	16	18	18	16	17	61	69	71	63	65	66
Do	7.50	20	16	13	16	16	16	59	61	66	63	56	61
Ferric citrate	8	24	21	24	22	24	23	63	64	65	71	64	65
Solution of ferric citrate	....	24	20	25	28	26	25	75	75	79	77	69	75
Ferric tartrate	9.34	27	20	22	24	21	23	71	69	74	66	62	68
Solution of ferric tartrate	....	23	26	15	25	26	23	67	78	72	75	57	70
Tobacco stems	40	28	30	26	33	31	30	62	64	66	72	67	66
Do	80	36	32	32	37	32	34	57	56	58	64	63	60
Stizolobium vines	40	24	26	24	32	25	26	74	60	68	66	70	68
Do	80	38	34	37	32	34	35	67	65	66	67	68	67
Dried blood	4	21	22	19	21	24	21	65	65	69	78	76	71
"Mixture"	92	28	27	26	30	26	27	59	54	57	59	56	57
Citric acid	10	29	23	32	32	23	28	72	68	66	58	77	68
Tartaric acid	10	28	33	26	36	28	30	71	67	71	68	73	70

After three weeks the plants in noncalcareous soil were about twice the size of those in calcareous soil. Later the plants in calcareous soil were all more or less chlorotic, but the plants in pots receiving the larger applications of tobacco stems, cover crop, or "mixture" were less chlorotic than others. All the plants in the noncalcareous soil were a good green throughout growth.

In the noncalcareous soil none of the materials significantly affected growth except the "mixture," which depressed the yield about 20 per cent. In the calcareous soil the "ferric humate" was distinctly injurious, while the larger applications of tobacco stems and Stizolobium vines were plainly beneficial, although they did not induce a normal growth.

SUMMARY.—All organic iron compounds tried in the two preceding experiments failed to increase appreciably the growth of rice in the calcareous soils. It is, therefore, probable that organic iron is no more available than inorganic iron in such soils.

While concentrated or soluble organic materials, such as dried blood, citric and tartaric acids, molasses, and a humus extract, failed to ameliorate the chlorosis, bulky organic materials, such as tobacco stems and velvet bean plants, when used in considerable quantities measurably improved the growth and color of the plants. Also, in previous work with pineapples and sugar cane large amounts of stable manure ameliorated or completely overcame the chlorosis, although small amounts were without appreciable effect.

In view of the nonavailability of the concentrated organic iron compounds, it seems probable that the beneficial effect of the bulky organic materials was not due primarily to the addition of certain iron compounds that were available in the calcareous soil as a whole. It is more probable that the particles of organic material formed isolated centers or points where iron was more available than in the rest of the soil. The plants were not able to secure all the iron they needed from these points for the reason that plants are apparently not able to absorb a maximum amount of iron with only a portion of their roots (*18*).

It may seem that the results of the last two tests negative the conclusions arrived at in the experiments with rice grown in solutions containing carbonate of lime where organic iron compounds supplied sufficient available iron. Conditions in the nutrient solutions, however, were somewhat different from those in the soil. To begin with, in the nutrient solutions the plants obtained their iron from an ordinary solution that was more or less sterile and that was frequently renewed. In the soil, on the other hand, the plants probably obtained their nutrients from aqueous films surrounding the soil particles, and there is evidence that in films reactions may occur which do not take place in ordinary solutions. Furthermore, bacterial action in the soil might have destroyed rapidly certain of the organic compounds supplied.[1]

EFFECT OF WATER CONTENT OF SOIL ON THE AVAILABILITY OF IRON

At present we know little of the true soil solution or film moisture. It is evident, however, that the nature of the soil particles must influence the composition of the solution or substances dissolved in the enveloping film. In the films surrounding particles of calcium carbonate the amount of iron in solution must be greatly reduced, since the iron would be precipitated as ferric oxid.

If it is assumed that each particle in the soil is isolated and that the moisture films surrounding the individual particles are discontinuous, it would follow that the larger the proportion of particles which were carbonate of lime the less soluble iron there would be in the whole medium.

This assumption would explain why carbonate of lime is more effective in inducing chlorosis the more finely divided it is and why a certain quantity of carbonate of lime exerts a stronger influence in a sandy soil containing relatively few particles than in a clay soil containing a large number of particles.

However, the case is not so simple as is assumed above. The moisture films are not discontinuous but more or less continuous, the continuity

[1] The fact that ferric citrate and ferric tartrate were no more effective when applied in frequent small doses than when applied all at once is some evidence against the idea that the organic iron compounds were unavailable because they were destroyed by bacterial action.

and thickness of the films depending somewhat on the amount of moisture in the soil. The substances in solution in a film surrounding one particle will therefore react with those in films surrounding adjacent films. One particle of carbonate of lime would affect the soluble iron in the films of a certain number of adjacent particles.

While the moisture films are to a certain extent continuous, we know that the composition of the films is not uniform throughout the soil. This is evident from certain well-established facts, such as the slight lateral movement of fertilizers. If the composition of the films were uniform and conditions were analogous to those in a solution with relatively few solid particles, a slight amount of carbonate of lime would have the same effect as a much larger amount. This, however, is not the case.

It might be expected that the effect of carbonate of lime in depressing the availability of iron and in inducing chlorosis would be influenced somewhat by the amount of water in the soil, since the aggregation of the soil particles and their moisture films would be affected by the water content. It was, therefore, of interest to observe the manner in which the growth and chlorosis of rice would be affected by different percentages of moisture in calcareous soil.

A preliminary test was conducted with four pots, each of which held 36 pounds of soil containing 15 per cent of calcium carbonate. Twelve rice plants were grown in each pot with abundant fertilizer. The plants were grown 30 days with 22 per cent of moisture in the soil. Water was then added to two of the pots until there were 2 inches of water above the surface of the soil, and the other two pots were maintained unchanged at 22 per cent moisture. After 67 days' growth the plants were cut.

The plants in all four pots were very slightly chlorotic at 30 days, but a few days after the extra water was added the submerged plants became intensely chlorotic and remained so for about 10 days. They then quickly improved in color, and a few days later the submerged plants were a perfectly normal green, while the plants in the soil with 22 per cent moisture were markedly chlorotic. This difference persisted until the plants were cut. The plants grown for the whole period with 22 per cent moisture gave an average green weight of 175 gm. per pot, while the plants grown for 30 days with 22 per cent moisture and then submerged for 37 days yielded 424 gm. per pot.

Experiment X.—An extended test was conducted from January 2 to March 22, 1918, using one noncalcareous soil and two calcareous soils (one a beach sand with practically no organic matter and the other a loam).[1] The noncalcareous soil was used as a control to determine how the growth of rice would be affected by different amounts of water in a

[1] The calcareous loam was the same as the noncalcareous soil except for the addition of the carbonate of lime some years before.

soil adapted to its growth. Each pot received 9 gm. sulphate of potash, 6 gm. double superphosphate, and 22.5 gm. sulphate of ammonia divided in two applications. Twenty rice plants were planted in each pot, but these were thinned to 10 when growth was well established. The results are given in Table XIII.

TABLE XIII.—*Effect of varying degrees of moisture on the availability of iron to rice plants in calcareous and noncalcareous soils*

Soil No.	Kind of soil.	Percentage of calcium carbonate.	Optimum water content of soil expressed as percentage of dry weight of soil.	Maximum water capacity of soil expressed as percentage of dry weight of soil.	Amount of soil per pot.	Amount of water maintained in soil during growth of plant.	Oven-dried yield of plants per pot.			
							Series A.	Series B.	Series C.	Average.
					Pounds.		*Gm.*	*Gm.*	*Gm.*	*Gm.*
1647	Loam	....	25.5	34.3	69	22.3 per cent	119.8	122.1	103.9	115.3
						26.3 per cent	125.4	120.8	127.0	124.4
						30.3 per cent	146.1	128.9	137.7	137.6
						34.3 per cent	129.2	142.1	141.6	137.6
						Water at surface of soil	159.9	167.5	153.8	160.4
						Water 3 inches above surface of soil	155.9	157.0	177.6	163.8
1648	...do...	8.53	23.2	36.2	69	20.2 per cent	58.1	51.5	57.5	55.7
						24.2 per cent	74.9	68.9	79.1	74.3
						28.2 per cent	53.7	70.8	78.8	67.8
						32.2 per cent	66.1	74.2	67.1	69.1
						36.2 per cent	87.3	72.9	77.8	79.3
						Water 3 inches above surface of soil	112.5	134.6	122.8	123.3
1194	Sand	19.0	11.6	25.0	98	11 per cent	12.4	9.9	9.2	10.5
						18 per cent	6.1	13.4	13.6	11.1
						25 per cent	1.2	9.4	1.4	4.0
						Water 3 inches above surface of soil	17.6	8.4	28.1	18.1

The different water contents maintained during the experiment were made up when the plants were 4 days old, except that the pots to receive 3 inches excess water were made up with water at the surface at this time, the water being raised to 3 inches when growth permitted it. When 11 days old, the plants in soils No. 1647 and 1648, where water was at the surface or above it, were markedly chlorotic, as well as all the plants in soil No. 1194. After 31 days' growth, all the plants in soil No. 1194 were still markedly chlorotic; the submerged plants in soil No. 1647 were normal green and were growing rapidly, as were all other plants in this soil; in soil No. 1648 the submerged plants and those in pots with 20.2 and 24.2 per cent water were normal green, while those in pots with 28.2, 32.2, and 36.2 per cent water were plainly chlorotic. At 72 days' growth, when the plants were cut, the appearance in regard to chlorosis was similar to that at 31 days, except that in soil No. 1194 the few plants that had not died in the pots with 3 inches excess water were normal green and far larger than the others.

The temporary chlorosis affecting the plants where the excess water was added is entirely distinct from the lime-induced chlorosis. A similar yellowing takes place in the field when the fields are flooded following early growth without submergence. Several of the surplus plants in the pots with excess water were brushed repeatedly with ferrous sulphate, but the treatment did not improve the color of the plants in the slightest. Evidently this particular chlorosis is not due to lack of iron. Doubtless when the water content of the soil is raised above the point of saturation the old roots are unable to function properly and the nutrition of the plant is disturbed until new roots are sent forth which are able to function under the new conditions.

It was thought that roots of the submerged plants might show morphological differences from roots of plants grown with ordinary amounts of water in the soil. Samples of roots from plants grown in soil No. 1647 were therefore subjected to a preliminary examination by Dr. Albert Mann, of the Bureau of Plant Industry, United States Department of Agriculture, to whom thanks are due. A portion of Dr. Mann's report of the preliminary examination follows:

> The differences noted between No. 1805 with 24.2 per cent moisture, 1807 with 32.2 per cent, and 1809 with water standing three inches above the surface are slight. There is in general more compactness and strength of tissue in 1805 than in the others. The central fibrovascular bundle mass is larger in proportion to the cortex than in 1807 or 1809. The cells of all the tissues are slightly more robust. The light parenchyma, which makes up the cortex from the endodermal ring to the epiderm, is especially thinner walled and more developed in 1809. There is also a notable absence of root hairs in this sample as compared with the other two, which is, of course, the inevitable result of the roots growing submerged in water.

The series in the noncalcareous soil shows that the growth of rice should increase regularly with increasing amounts of water in the soil until a percentage near the saturation point of the soil is reached and that, possibly because of a different root growth, there should be another considerable increase when enough water is added for submergence. In No. 1648, however, the series with the calcareous soil, there were two maxima of growth, one at 24.2 per cent water and one at 3 inches excess; and in the calcareous sand No. 1194 there were also two maxima. It is believed that the first lower maximum was due to iron being a little more available at that water content than at a higher content. The great increase in growth in the calcareous soils produced by submergence [1] was probably due chiefly to the fact that the modified roots are better able to assimilate iron than the ordinary type of root and was probably not due to increased availability of iron in the submerged soil.

It is felt that the results substantiate the idea that the availability of iron in the soil is affected somewhat by the amount of water in the soil, the availability being slightly greater near the optimum water content than with larger amounts.

The effect of the water content is probably due to its influence on the extent to which reactions take place between the moisture films

[1] It will be noted that in the calcareous soils the increase produced by submergence was much greater than in the noncalcareous soil.

surrounding the calcareous particles and those surrounding the other soil particles. With moisture contents above the optimum the moisture films become more continuous and the sphere of influence of the particles of carbonate of lime in reducing the availability of iron becomes more extended.

Incidentally the tests established a fact of considerable practical importance—namely, that rice may be expected to make a practically normal growth in certain calcareous soils if the soils are submerged.

SUMMARY

There are a few plants which are generally conceded to be calcifugous, inasmuch as they are rarely found on calcareous soils.

Soil surveys of several species of cultivated plants show that a particular type of chlorosis affecting these plants occurs only on calcareous soils. All calcareous soils, however, do not induce chlorosis in these plants.

Addition of carbonate of lime to soils producing normal, calcifugous plants causes the soils to produce chlorotic plants.

It is, therefore, evident that a chlorosis of some plants is caused by, or is associated with, the presence of carbonate of lime in the soil.

The weight of the evidence from ash analyses of chlorotic plants seems to point to a deficiency of iron in the ash as being one cause of the chlorosis, with possibly an excess of lime as a contributory cause.

Treatment of chlorotic plants with iron shows that a lack of iron in the plant is at least one of the causes of lime-induced chlorosis.

There is no evidence of a general "lime effect" in inducing chlorosis, the different lime compounds affecting the plants differently.

Rice, one of the plants sensitive to lime, does not appear to be sensitive to the alkalinity of carbonate of lime except as this alkalinity influences the availability of the iron.

Lime-induced chlorosis seems to be due simply to a depression in the availability of iron in calcareous soils.

A number of pure organic iron compounds and concentrated organic preparations proved to be inefficient sources of iron for rice in calcareous soils. Bulky organic compounds such as stable manure, velvet bean plants, and tobacco stems, when used in considerable quantity, however, enabled the plant to secure more iron.

The availability of iron in calcareous soils appears to be slightly greater near the optimum water content of the soil than at higher percentages of water.

Although rice becomes chlorotic in calcareous soils with ordinary percentages of water, it will grow normally in certain calcareous soils if the soil is submerged. This is believed to be due to the growth, under submerged conditions, of a new kind of root that is better able to assimilate iron than the root formed in the soil with less water.

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PLATE 5

A.—Rice grown in calcareous and noncalcareous soils and sprayed with ferrous sulphate solution (experiment I).

1–4. Noncalcareous soil; plants in 1 and 3 unsprayed, those in 2 and 4 sprayed.

5–8. Soil containing 30 per cent carbonate of lime; plants in 5 and 7 unsprayed, those in 6 and 8 sprayed.

B.—Apparatus used in growing plants in experiment VII.


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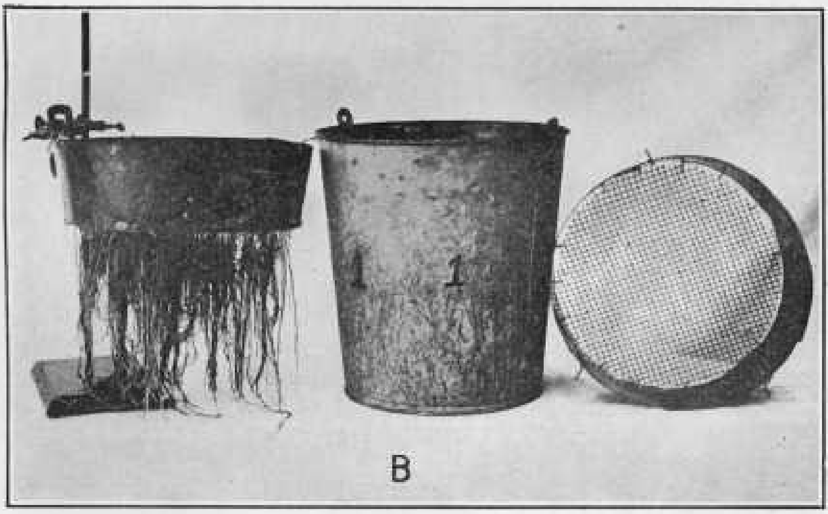
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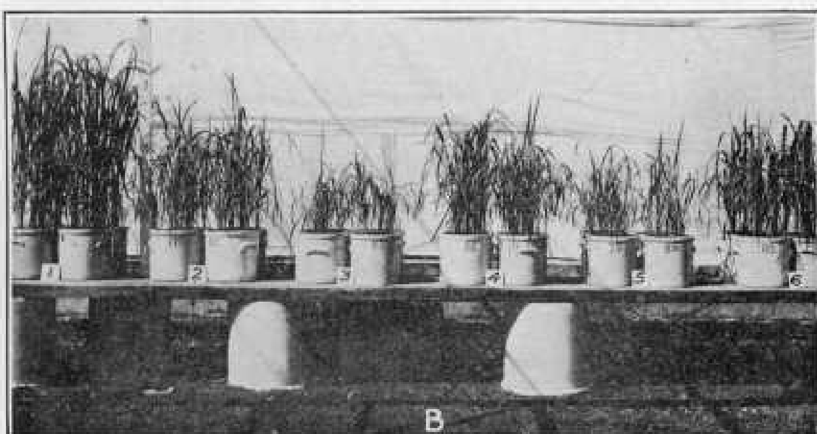
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PLATE 6

A.—Effect of carbonate of lime in depressing the availability of iron (experiment VII).

1. Calcareous soil in bucket, silica sand plus iron in sieve.
2. Calcareous soil in bucket, silica sand plus carbonate of lime and iron in sieve.

B.—Effect of various substances on growth of rice in calcareous soil (experiment VIII).

1. Noncalcareous soil.
2. Calcareous soil.
3. Calcareous soil with molasses added.
4. Calcareous soil with "ferric molasses" added.
5. Calcareous soil with molasses and ferrous sulphate added.
6. Calcareous soil with ferrous sulphate added.